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Characterization of Saline Soil Rhizobacteria from Coastal Lands in Dissolving Phosphate, Nitrogen Fixing and Synthesizing IAA Growth Hormone

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Saline soils are a major constraint to agricultural productivity, affecting nutrient availability and plant growth due to high salt concentrations. As global agricultural demands increase, sustainable strategies are needed to improve crop resilience and productivity in saline environments. Rhizobacteria, particularly those with the ability to solubilize phosphate, fix atmospheric nitrogen, and produce plant growth hormones such as indole-3-acetic acid (IAA), offer a promising solution. The study aimed to identify potential indigenous rhizobacteria extracted from the rhizosphere of saline soil in Southeast Sulawesi. The research was carried out in the Agronomy Laboratory, Faculty of Agriculture Halu Oleo University. The experimental design employed a completely randomized setup, involving ten different isolates. These isolates were assessed for their capacity to solubilize phosphate, fix nitrogen, and produce the growth hormone known as IAA. The findings revealed that all the rhizobacteria isolates exhibited the ability to fix nitrogen, and synthesize IAA, however, 2 isolates could not dissolve phosphate. Among them, the rhizobacteria labeled KLK-LS14 displayed the highest phosphate solubilization, with a halo diameter of up to 1.55 cm. As for nitrogen fixation, the isolates KDI-LS04, KNW-LS08, KLK-LS10, and KLK-LS14 demonstrated the highest levels. In terms of IAA hormone synthesis, the isolates KNW-LS08, KLK-LS14, and KDI-LS04 exhibited the greatest production, with respective contents of 47.44 µg/ml filtrate, 48.11 µg/ml filtrate, and 50 µg/ml filtrate. The most promising isolates, such as KLK-LS14 and KNW-LS08, exhibited high nitrogen fixation and IAA production, making them suitable candidates for agricultural bio-inoculants. These findings suggest practical applications in sustainable agriculture, particularly for reclaiming saline soils. Future work should focus on large-scale field trials and integrating these rhizobacteria into farming practices to improve crop yield, reduce dependency on chemical fertilizers, and support the development of environmentally friendly agricultural technologies.

Keywords: Indigenous rhizobacteria, IAA, phosphate solvent, nitrogen fixer, saline soil, sustainable agriculture, Plant Growth-Promoting Rhizobacteria (PGPR).

INTRODUCTION

The increasing global emphasis on sustainable agriculture has highlighted the importance of environmentally friendly strategies to enhance crop productivity. In this context, Plant Growth Promoting Rhizobacteria (PGPR) have garnered significant attention for their role in promoting plant growth while reducing dependence on chemical inputs. inputs (Molina-Montenegro *et al*., 2020; Sharma *et al*., 2016). These microorganisms, inhabiting the rhizosphere, exhibit multifunctional traits such as phosphate solubilization, nitrogen fixation, and production of plant growth hormones

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like indole-3-acetic acid (IAA), directly and indirectly influencing plant development (Sharma *et al*., 2016; Bharti *et al*., 2016). Notably, PGPRs such as *Pseudomonas spp.* and *Bacillus spp.* have been shown to increase nutrient availability, enhance plant growth, and improve tolerance to biotic and abiotic stresses, including salinity (Sharma *et al*., 2016; Bharti *et al*., 2016; Acuña-Rodríguez *et al*., 2019).

In accordance with the ongoing agricultural sector's emphasis on environmentally friendly and sustainable practices, this research serves as a viable solution. The rhizosphere of every patch of soil harbors countless potential rhizobacteria that can be harnessed as Plant Growth Promoting Rhizobacteria (PGPR). It is believed that differences in the host plants where these rhizobacteria thrive can result in variations in their biochemical characteristics. Specifically, these biochemical traits are linked to their ability to stimulate plant growth by means of phosphate solubilization, nitrogen fixation, and the synthesis of the growth hormone known as IAA.

Numerous similar studies have been conducted not only in Indonesia but also worldwide. The outcomes have exhibited variability, influenced by the specific crop type and the environmental conditions in which the rhizobacteria were investigated. Previous studies have shown that PGPR rhizobacteria are commonly found in genera such as *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp. (Guyasa *et al*., 2018; Sutariati *et al*., 2018a), *Azospirillum* spp., *Azotobacter* spp., and *Enterobacter* spp. (Souza *et al*., 2015). Generally, these types of bacteria possess the capacity to enhance plant growth and yield while also providing protection against various diseases (Ilyas *et al*., 2015; Yasmin *et al*., 2016; Radhakrishnan *et al*., 2017).The capacity of rhizobacteria to perform tasks such as phosphate solubilization, nitrogen fixation, and growth hormone synthesis has a dual impact, both direct and indirect, on the enhancement of plant growth and yield (Sutariati *et al*., 2018b). Additionally, Govindasamy *et al*. (2016) have asserted that the role of PGPR in boosting plant growth and yield is associated with their ability to produce growth hormones, mobilize nutrients by dissolving phosphorus, fix nitrogen, or indirectly contribute by mitigating biotic stress, such as activating plant defense mechanisms against disease-causing pathogens.

The salinity of soils in coastal regions poses a major challenge to sustainable agriculture, limiting the availability of essential nutrients and impairing plant growth (Nawaz *et al*., 2020; Kumar *et al*., 2020; Arora *et al*., 2020; Hanin *et al*., 2016; Cai *et al*., 2021). Addressing this issue requires the identification of biological solutions that enhance nutrient cycling and improve plant productivity (Nawaz *et al*., 2020; Upadhyay *et al*., 2011; Oliva *et al*., 2023; Latef *et al*., 2021; Zafar-ul-Hye *et al*., 2019; Saidi *et al*., 2021). The use of PGPR, particularly those capable of solubilizing phosphate, fixing nitrogen, and synthesizing IAA, represents a promising approach (Nawaz *et al*., 2020; Upadhyay *et al*., 2011; Latef *et al*., 2021; Sharma *et al*., 2021; Saidi *et al*., 2021, Javed *et al*., 2020; Egamberdieva *et al*., 2019). By exploring the rhizosphere of plants in saline soils, this study seeks to identify indigenous rhizobacteria with these beneficial traits, offering a sustainable alternative to chemical inputs for improving crop yields in marginal land conditions (Nawaz *et al*., 2020; Upadhyay *et al*., 2011; Oliva *et al*., 2023; Latef *et al*., 2021; Sharma *et al*., 2021, Irum, 2023; Zafar-ul-Hye *et al*., 2019; Cordero *et al*., 2018; Omara *et al*., 2022; Redondo-Gómez *et al*., 2021).

It has been demonstrated that the inoculation of seeds with PGPR rhizobacteria through biopriming treatments can enhance plant growth while suppressing the growth of pathogenic organisms (Liu *et al*., 2017). For example, a study conducted by Shakeel *et al*. (2015) on rice seeds revealed that the inoculation of rice seeds with *Bacillus* sp. led to a notable increase in rice growth and yield, ranging from 22% to 49%. Similarly, Elektyar *et al*. (2015) reported that rice seed inoculation with *Pseudomonas fluorescens* resulted in a remarkable production increase of up to 47%.

However, the efficiency of these PGPR traits often varies depending on the isolate's origin and environmental adaptation. Saline soils, characterized by high osmotic pressure and limited nutrient availability, present unique challenges for microbial survival and functionality. While several studies have evaluated PGPR in general agricultural settings, research focusing on rhizobacteria from saline soils remains limited (Anti *et al*., 2020; Nawaz *et al*.. 2020; Wang *et al*., 2014). Moreover, most studies emphasize laboratory-scale evaluations, necessitating further investigations to determine their efficacy under field conditions (Mahmood *et al*., 2017; Irum, 2023; Hahm *et al*., 2017). These gaps highlight the need to explore indigenous rhizobacteria from saline environments and assess their potential as bioinoculants for enhancing crop productivity.

The study identifies high-performing saline soil rhizobacteria isolates for sustainable agriculture, enhancing nutrient availability and crop productivity in saline environments. In the context of this study, an initial selection process involved the identification of 200 rhizobacterial isolates from the rhizosphere of areca plants cultivated in marginal lands (Ultisols) without the use of fertilizers. From this initial selection, the top 10 most promising isolates were chosen for further evaluation. This research serves as a subsequent step in the selection process, aiming to identify the most suitable isolates for use as PGPR, particularly for agricultural commodities cultivated in marginal land conditions.

MATERIALS AND METHODS

***Rhizobacterial isolates*:** The rhizobacteria isolates used were the result of selection from previous research stages (Sutariati *et al*., 2020), consisting of 10 isolates.

***IAA synthesis assay*:** The ability of rhizobacterial isolates to synthesize IAA was analyzed using the method of Glickman

and Dessaux (1995). The content of IAA in the samples was calculated by regression made of pure IAA at concentrations of 0, 6.25, 12.5, 25, 50, 75, 100, 150 and 200 µg.ml^{-1}.

***Phosphate dissolution test*:** To assess the capacity of rhizobacteria to solubilize phosphate, a test was conducted using an insoluble dicalcium phosphate (DCP) medium. The testing protocol followed the method described by Goldstein in 1986, as outlined in Munif's work in 2001. Rhizobacterial isolates demonstrating the ability to dissolve phosphate were identified by the presence of a distinct halo (clear zone) surrounding the well containing the bacterial suspension.

***Nitrogen fixation test*:** To qualitatively analyze bacterial endorhizal isolates for their ability to fix atmospheric nitrogen, the following procedure was employed using Burk Salt media: A Burk Salt media stock was prepared by mixing 2 grams of $MgSO_4$, 8 grams of K_2HPO_4, 2 grams of KH_2PO_4, and 1.3 grams of $CaSO_4$. A stock solution of Fe-Mo was created by dissolving 0.145 grams of $FeCl_3$ and 0.0235 grams of Na_2MoO_4 in 100 ml of distilled water. To make the Burk Salt medium, 1.3 grams of the Burk Salt medium was mixed with 1 ml of the Fe-Mo stock solution, and then 10 grams of sucrose were added. All these components were dissolved in 1000 ml of sterile distilled water and sterilized using an autoclave at 121°C under a pressure of 1 atmosphere for 15 minutes. Next, 20 µl of the Burk Salt medium was dispensed into a sterile test tube. A single dose of the rhizobacterial isolate under test was introduced into the solution. The test tubes were then placed in an incubator shaker and incubated for 48 hours at 150 rpm. To determine if an isolate is a nitrogen fixer, the presence of turbidity in the Burk Salt solution within the test tube was observed. Isolates showing bacterial growth were marked as "+" (positive), while those not exhibiting growth were labeled as "-" (negative).

***Propagation of rhizobacteria isolates*:** Pure cultures of bacterial endo-rhizo isolates were propagated using TSA media for the *Bacillus* sp. group. and for the *Pseudomonas* sp group. King's B media was used. Growing bacterial colonies were suspended in a suitable liquid medium until they reached a population density of 10^9 cfu ml^{-1} (Bai *et al*., 2002).

***Data analysis*:** Statistical analyses were performed using two-ways of analysis of variances (ANOVA) by means using the Statistical Package of Social Sciences (SPSS) program version 20 for Windows (Chicago, IL, USA). If the test result showed a significant difference, then tests of treatment differences were performed using Duncan's Multiple Range Test (DMRT) at α=0.05.

RESULTS AND DISCUSSION

***Ability of rhizobacteria to dissolve phosphate and nitrogen fixation*:** The rhizobacteria isolates from the saline soil tested had different phosphate-dissolving abilities, and not all isolates could dissolve phosphate (two isolates could not dissolve phosphate). On the other hand, all isolates tested were able to fix free nitrogen from the air. Rhizobacteria group *Pseudomonas* spp. capable of dissolving phosphate with a halo diameter range of 1.15-1.40 cm, while the *Bacillus* spp. capable of dissolving phosphate with a halo diameter of 0-1.25 cm. On the other hand, the rhizobacteria of the *Pseudomonas* spp. able to fix N with categories from slightly cloudy to very cloudy, while the *Bacillus* spp. able to fix N in the very turbid category. Similarly, in fixing N, rhizobacteria of the *Pseudomonas* spp. able to fix N with categories from slightly cloudy to very cloudy, while the *Bacillus* spp. able to fix N with cloudy category (Table 1). Differences in exploration areas also influence the ability of rhizobacteria to solubilize phosphate and fix N (Table 1). The performance of the turbidity level of the Burk salt solution as an indicator of the ability of rhizobacteria to fix nitrogen can be seen in Figure 1.

***Synthesis of IAA by rhizobacteria*:** Similar to the previous explanation, where all types of bacteria tested demonstrated the capability to solubilize phosphate and fix atmospheric nitrogen, the findings of this research also indicate that all

Table 1. Ability of rhizobacteria isolates from the *Bacillus* spp., or *Psedomonas* spp. in dissolving phosphate and fixing N.

Bacteria Group	Isolates code	Phosphate solvent (halo diameter=cm)	Fixation N	Isolate Origin (Regency)
Bacillus spp.	KNU-LS02	-	++	North Konawe
Pseudomonas spp.	KNU-LS08	1.15	++	North Konawe
Bacillus spp.	KNU-LS13	1.25	+++	North Konawe
Pseudomonas spp.	KDI-LS04	1.20	++++	Kendari
Pseudomonas spp.	KDI-LS07	1.40	+++	Kendari
Pseudomonas spp.	KNW-LS08	1.25	++++	Konawe
Bacillus spp.	KNW-LS09	1.25	++	Konawe
Bacillus spp.	KLK-LS06	-	++	Kolaka
Pseudomonas spp.	KLK-LS10	1.30	++++	Kolaka
Pseudomonas spp.	KLK-LS14	1.55	++++	kolaka

Description: **(slightly cloudy), ***(turbid), ****(very cloudy); Description: ++++ = Very Cloudy, +++ = Cloudy ++ = Moderately Cloudy, - = Not Cloudy

tested bacteria, encompassing both endophytic and rhizobacterial strains, possessed the capacity to produce the growth hormone IAA when cultured in media containing tryptophan (Figure 2).

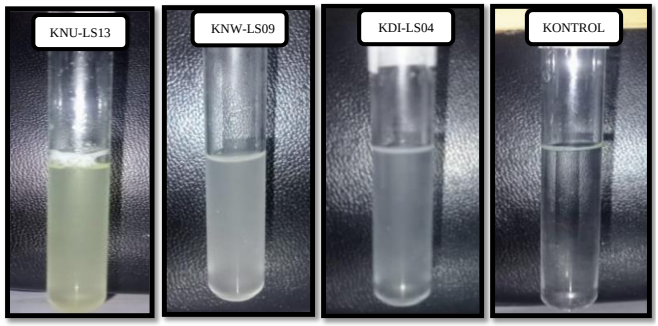

Figure 1. The ability of rhizobacteria to fix nitrogen in Burk salt solution.

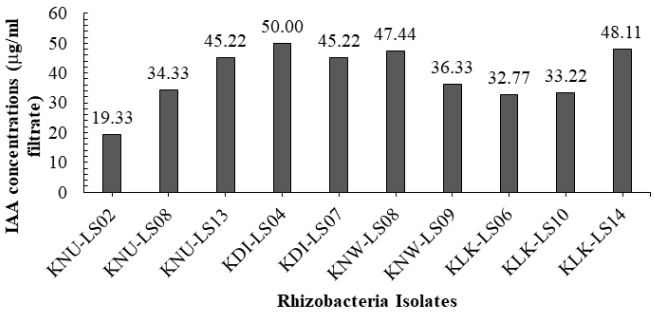

Figure 2. Ability of bacteria to synthesize the growth hormone IAA in media containing tryptophan.

The results revealed that the rhizobacteria exhibited IAA synthesis abilities within a concentration range of 19.33 to 50.00 µg/ml filtrate. Furthermore, the study's outcomes indicated that *Pseudomonas* spp. bacteria exhibited a higher propensity for IAA synthesis compared to the *Bacillus* spp. group (as depicted in Figure 2).

DISCUSSION

The rhizobacteria isolates from the saline soil exhibited the capability to solubilize phosphate, as evidenced by the presence of a clear zone in the media containing the phosphate source. This research aligns with prior studies that have reported phosphate solubilization by both endophytic bacteria (Ikade *et al*., 2010; Jalgaonwala and Mahaja, 2011; Kaaria *et al*., 2012; Nongkhlaw and Joshi, 2014; Sutariati *et al*., 2019) and rhizobacteria (Anwar *et al*., 2014; Karpagam and Nagalakshmi, 2014; Gupta *et al*., 2015; Bouali *et al*., 2016; Babu *et al*., 2017; Guyasa *et al*., 2018; Sutariati *et al*., 2018c). It's worth noting that the ability to solubilize phosphate was generally more pronounced in the *Pseudomonas* spp. compared to the *Bacillus* spp. group, encompassing both endophytic bacteria and rhizobacteria. In accordance with the findings of this study, Guyasa *et al*. (2018) reported that *Pseudomonas* spp. bacteria were more effective at phosphate solubilization in TCP media when compared to *Bacillus* spp. Pande *et al*. (2017) also highlighted that variations in the ability of bacterial isolates to solubilize phosphate were influenced by several factors, including the type of phosphate source used, media pH, temperature, incubation duration, and the carbon and nitrogen sources employed.

Karpagam and Nagalakshmi (2014) reported the presence of 37 phosphate-solubilizing microbial isolates on Pikovskaya agar medium, evidenced by the formation of clear zones in the medium around insoluble tricalcium phosphate (TCP). Among these isolates, 8 strains belonging to the genera *Pseudomonas* sp., *Bacillus* sp., and *Rhizobium* sp. displayed the highest phosphate dissolution indices, ranging from 1.13 to 3.0. Niswati *et al*. (2008) elucidated that phosphate-solubilizing bacteria (BPF) facilitate the conversion of organic and inorganic phosphates into soluble forms by producing organic acids. These organic acids can replace phosphorus in bonds with aluminium or iron, consequently releasing phosphorus into a soluble form accessible to plants. Hasanuddin and Bambang (2004) further elaborated that the rhizosphere harbors a substantial number of microorganisms known to solubilize and provide insoluble phosphorus in a form usable by plants.

In addition to their phosphate-solubilizing abilities, rhizobacteria isolates displayed nitrogen-fixing capabilities, as indicated by the turbidity levels observed in Burk Salt media. These findings are consistent with previous research demonstrating the nitrogen-fixing capacity of bacteria, including both endophytic bacteria (Doty *et al*., 2009; Duangpaeng *et al*., 2012; Kaaria *et al*., 2012; Nongkhlaw and Joshi, 2014; Sutariati *et al*., 2019) and rhizobacteria (Gupta *et al*., 2015; Guyasa *et al*., 2018; Sutariati *et al*., 2018c).

Among the rhizobacteria isolates, those from the *Pseudomonas* spp. demonstrated the ability to fix nitrogen, ranging from slightly turbid to very turbid categories. *Bacillus* spp. isolates were also capable of fixing nitrogen, with a classification of "very turbid." Moreover, endophytic bacteria from the *Pseudomonas* spp. group exhibited nitrogen-fixing abilities ranging from slightly turbid to very turbid, while *Bacillus* spp. displayed a "cloudy" category for nitrogen fixation. It is important to note that the differences in exploration areas may influence the nitrogen-fixing abilities of rhizobacteria.

In addition to their roles as phosphate solubilizers and nitrogen fixers, the isolates of both endophytic bacteria and rhizobacteria exhibited the capacity to produce growth hormones in the form of IAA across various tryptophan-containing media. The findings revealed that rhizobacteria displayed a broader concentration range for IAA synthesis, spanning from 5.45 to 71.27 µg/ml filtrate, whereas endophytic bacteria exhibited a narrower concentration range, ranging from 26.36 to 67.00 µg/ml filtrate.

Moreover, the study results were consistent with previous research indicating that bacteria from the Pseudomonas sp. exhibited a higher proficiency in synthesizing the IAA hormone when compared to other bacterial groups (Guyasa *et al*., 2018). Reetha *et al*. (2014) also reported that IAA production by *Bacillus* spp. was lower when compared to the IAA production by *P. fluorescens*.

***Conclusion*:** The findings revealed that all the rhizobacteria isolates exhibited the ability to fix nitrogen, and synthesize IAA, however, 2 isolates could not dissolve phosphate. Among them, the rhizobacteria labeled KLK-LS14 displayed the highest phosphate solubilization, with a halo diameter of up to 1.55 cm. As for nitrogen fixation, the isolates KDI-LS04, KNW-LS08, KLK-LS10, and KLK-LS14 demonstrated the highest levels. In terms of IAA hormone synthesis, the isolates KNW-LS08, KLK-LS14, and KDI-LS04 exhibited the greatest production, with respective contents of 47.44 µg/ml filtrate, 48.11 µg/ml filtrate, and 50 µg/ml filtrate. Further research is necessary to assess the effectiveness of these isolates in enhancing plant growth and yield on a larger scale in field conditions.

***CRediT author statement*:** G.A.K. Sutariati, Muhidin, K. Amartani, I.M. Guyasa designed, complete the experiments; A. Bahrun, N.M. Rahni, G.R. Sadimantara, T.C. Rakian, L. Mudi prepared the draft; G.N.A. Wibawa, A. Madiki reviewed and finalized the draft.

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***SDGs addressed*:** Zero hunger, Responsible Consumption and Production, Climate Action and Life on Land.

***Policy referred*:** Sustainable Agriculture Focus, Saline Soil Reclamation, Environmental Protection and Climate Adaptation, Future Integration with Farming Practices.

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